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13. ABSTRACT *(Maximum 200 words)*

The focus of the first years activities, at a time when a major recruitment of personnel was underway, revolved around: selection of the appropriate cell line for performing the mammalian cell mutagenicity studies; preliminary studies with the Balb/c 3T3 cell transformation assay; mutation spectrum analysis of the spontaneous mutants arising in AS52 and BH4 chinese hamster ovary cell lines; liposome encapsulation studies of the polymer diazoluminomelanin (DALM); and redesign of the thermal control system originally constructed as a prototype for the Radiofrequency Radiation Division at USAF Armstrong Laboratory. Most importantly, although the original proposal called for the study of the possible mutagenic interaction of microwaves and ionizing radiation using the AS52 line of CHO cells, the results of the mutation spectrum analysis study led to the decision to alter future protocols to perform these studies with the BH4 cells; otherwise, the experiments would predominantly be measuring small deletion type mutations, and would not have enough sensitivity to pickup alterations of other types of mutation due to the microwave exposures. In addition, the liposome studies revealed the difficulty of encapsulating chemically synthesized DALM in this biological system; these studies will need to be furthered using other resources. The redesign and construction of a modified thermal control system was begun, allowing for one control unit to perform the temperature measurement and temperature control functions in two separate incubator systems.

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REPORT

CELLULAR AND MOLECULAR LEVEL RESPONSES AFTER RADIOFREQUENCY RADIATION EXPOSURE, ALONE OR IN COMBINATION WITH X-RAYS OR CHEMICALS

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30 July, 1992

First Year Report for Period 1 April 1991 - 31 March 1992

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1. SUMMARY

The focus of the first year's activities, at a time when a major recruitment of personnel was underway, revolved around: selection of the appropriate cell line for performing the mammalian cell mutagenicity studies; preliminary studies with the Balb/c 3T3 cell transformation assay; mutation spectrum analysis of the spontaneous mutants arising in AS52 and BH4 chinese hamster ovary (CHO) cell lines; liposome encapsulation studies of the polymer diazoluminomelanin (DALM); and redesign of the thermal control system originally constructed as a prototype for the Radiofrequency Radiation Division at U.S. Air Force Armstrong laboratory. Most importantly, although the original proposal called for the study of the possible mutagenic interaction of microwaves and ionizing radiation using the AS52 line of CHO cells, the results of the mutation spectrum analysis study led to the decision to alter future protocols to perform these studies with the BH4 cells; otherwise, the experiments would predominantly be measuring small deletion type mutations, and would not have enough sensitivity to pick up alterations of other types of mutation which might occur due to the microwave exposures. In addition, the liposome studies revealed the difficulty of encapsulating chemically synthesized DALM in this biological system; these studies will need to be furthered using other resources. The redesign and construction of a modified thermal control system was begun, allowing for one control unit to perform the temperature measurement and temperature control functions in two separate incubator systems.

2. RESEARCH OBJECTIVES

The overall objective of this research will include the examination of four hypotheses:

I. That radiofrequency radiation (RFR), alone or in combination with ionizing radiation exposure or (selected) chemical treatment, will result in an effect either a) on proliferating mammalian cells, exposed either as asynchronous or synchronous cell populations; or b) non-proliferating mammalian cells, with this effect only being measureable during the period of RFR exposure;

II. That such an effect will be measureable for a transient period immediately after the RFR exposure ends;

III. That this effect may be measureable within the first two cell cycles post-exposure, after cellular metabolism leads to expression of the effect; or only at later times in the culture period of non-dividing cells; and

IV. That the effect will continue to be detectable in succeeding generations of dividing cells derived from the initially exposed parent cell population. The latter would result from DNA (genetic) alterations or cancer-like changes (transformation) of cells.

In examining these four major hypotheses, the project will investigate biological, biochemical, metabolic, structural, and genetic effects. These include studies of the effects on: cell survival by cloning efficiency or dye exclusion assay; cell growth by cell counting; DNA synthesis using flow cytometry; cell cycle distribution using flow cytometry; protein synthesis, with particular attention to stress proteins using electrophoresis; phosphate metabolism using NMR spectroscopy; membrane associate receptors using flow cytometry; mutation induction using selection of mutants with the chemical 6-thioguanine; and transformation using morphological examination of overgrown colonies. Because of concern for the possible effects on cells of the immune system, and the human immune system in particular, the cell line chosen for these studies was the 244B human lymphoblastoid cell line, which is an EBV-transformed

continuous "normal" cell line. All of the studies described are being performed with this cell line, with the exception of the mutation studies, which are being performed in BH4 and/or AS52 Chinese hamster ovary cells, and the transformation studies, which are being performed in Balb/c 3T3 cells. To the extent that DNA damage is suggested, later effects to be investigated include DNA damage and sister chromatid exchange induction.

3. STATUS OF RESEARCH

During the first year of this project, significant technical accomplishments were achieved, even in light of incomplete staffing. The latter was due to the departure from our laboratory at UTHSCSA of two of the scientists originally involved prior to the award of funding, and the difficulty encountered in recruiting replacements (see Section 8). Even with this situation, several aspects of the project were initiated and others completed during this project period.

a) Based on our expectation that the AS52 line of Chinese hamster ovary (CHO) cells would be more suited to our specific purposes than the BH4 CHO cell line, preliminary mutation experiments were performed with the former. The selection of the AS52 cell line was based on published reports that it was more sensitive to ionizing radiation induced mutation than the BH4 cells; i.e., mutations could be detected at lower levels of cell killing. This was important to us in regard to our objective of examining the possible mutagenic interaction between microwave radiation exposure and ionizing radiation exposure; we wanted to increase the mutation efficiency prior to any microwave exposure. As a result of our attention to more sophisticated analysis which we became aware of between the time of submission of the proposal and its initiation, we subsequently

arranged for a sub-contract with Dr. Abraham Hsie at the University of Texas Medical Branch at Galveston. This was approved by AFOSR. Dr. Hsie was asked to perform mutation spectrum analysis of the two different cell lines, AS52 and BH4. This information, for spontaneously occurring mutants, would in future work not only allow us to look at quantitative changes with the combined exposures, but qualitative changes as well. As is described below, the results were of a nature which subsequently led us to alter our original approach, and look to performing the quantitative mutation studies with the BH4 cell line. The use of the latter will allow for more sensitivity in detecting changes in different mechanisms (types) of mutation at the molecular level.

b) Through a subcontract, mutation spectrum analysis studies were performed at UTMB in the laboratory of Dr. Abraham Hsie. The results of these investigations are summarized below.

c) In scientific discussions with Dr. Johnathan Kiel of the U.S. Air Force Armstrong Laboratory, we became aware of his interest in attempting to deliver the chemical diazoluminomelanin (DALM) into mammalian cells. DALM was developed in his laboratory, and has unique microwave absorbing properties. The incubation of cells with DALM in solution was previously found to be unsuccessful, possibly due to the negatively charged polymer being repulsed by the surface charge on the cells. With the concurrence of Dr. Kiel, Dr. Beth Goins of the Division of Nuclear Medicine undertook an effort to encapsulate the DALM in liposomes; this is an alternative delivery system being investigated in many laboratories for its use in the chemotherapeutic treatment of mammalian cells. The research accomplished is summarized below.

d) As part of this project, we will be investigating the effects of individual and simultaneous microwave and ionizing radiation exposures on the

phosphate metabolism occurring in mammalian cells using nuclear magnetic resonance spectroscopy. In addition to measurement of the ATP and Pi ratio, we will be attempting to examine the effects on phospholipid metabolism. As a preliminary to performing the agar gel perfusion studies with the 244B human lymphoblastoid cells, an investigation was made of the number of acquisitions necessary for different concentrations of inorganic phosphate. This information is summarized below.

e) In order to avoid the problems inherent in using waterbaths in microwave exposure studies, and with the approval of Dr. Johnathan Kiel of the U.S. Air Force Armstrong Laboratory and AFOSR, we initiated the re-design and construction of a Thermal Control System similar to the prototype used in the Radiofrequency Radiation Division at U.S. Air Force Armstrong Laboratory. The modification allows for the independent control of two different incubators using one control unit. Some of the advantages are that two independent experiments can be performed at one time; or one incubator can be made to follow the temperature increase in the second; or the maximum temperature to be achieved can be pre-specified. The status of the instrumentation is described in Section 7.

Molecular Nature of Spontaneous Mutations at the hprt and gpt Genes in CHO Cells

The molecular nature of spontaneous and induced mammalian somatic cell mutations has been studied by investigating mutation spectra from a large number of mutants at the hprt and gpt loci in Chinese hamster ovary cells. Molecular techniques based on polymerase chain reaction (PCR) and direct sequencing analysis have been developed. These include multiplex DNA amplification of all nine exons for deletion detection in the hprt gene, direct hprt cDNA sequence analysis via reverse transcription and PCR amplification for determination of point mutations, and solid-phase direct exon sequencing for identification of RNA

splicing mutations. Mutation analysis at the gpt locus utilizes a simplified PCR method for deletion detection and direct sequencing for point mutation determination.

Analyses of 63 independent spontaneous hprt mutants have shown a variety of mutation events: single base substitutions (43%), deletions (24%), RNA splicing errors (24%), frameshifts (3%), and insertions and rearrangements (6%). Mutations are non-randomly distributed. Two hotspots for single base substitutions were found. The majority of deletion breakpoints (71%) were located in regions around exons 4, 5 and 6. RNA splicing mutations were found affecting exons 3 to 9, and mostly resulted in the loss of exon 7 (40%). Results from sequence analysis of 45 gpt mutants in AS52 cells have shown that 76% are deletions; either complete (53%) or partial (23%) gene deletions. The rest include base substitutions 11% and insertions (13%). The presence of hotspots for a 3-bp deletion in gpt gene is confirmed.

Large deletions in gamma-ray-induced hprt mutants are 76% (600 cGy) and 51% (400 cGy), indicating deletion proportions are affected by dose. Complete gene deletions are comprised of more than 90% of radiation induced mutants at the gpt locus. Among EMS-induced mutants, 95% and 84% are non-deletions at the hprt and gpt loci, respectively.

Table 1: Spontaneous HPRT Mutation Spectra in Chinese Hamster Cells

Total Mutants:	**63 (100%)**
1) Base Substitutions:	**27 (43%)ions:**
Transitions	
G:C->A:T	6
A:T->G:C	2
Transversions	
G:C->T:A	10
T:A->G:C	4
G:C->C:G	1
C:G->G:C	1
T:A->A:T	1
A:T->T:A	1
C:G->A:T	1
2) Deletions:	**15 (24%)**
Single Exon Deletions	
Exon 4	2
Exon 5	5
Exon 6	2
Multiple Exon Deletions	
Exons 1-5	1
Exons 6-9	2
Total Gene Deletion	1
Intragenic Deletions	2
3) RNA Splicing Mutations:	**15 (24%)**
Exon 3	1
Exon 4	2
Exon 5	2
Exon 6	2
Exon 7	6
Exon 8	2
4) Frameshift Mutations:	**2 (3%)**
Frameshift +1	1
Frameshift -1	1
5) Insertions/Rearrangements:	**6 (6%)**

Table 2: Multiplex PCR Deletion Screening at the HPRT Locus in Chinese Hamster Cells

Mutagen (Mutants analyzed)	Total Gene Deletion	Partial Gene Deletion	Insertion	No Alteration
Spontaneous (63)	1 (2%)	14 (22%)	2 (3%)	46 (73%)
Gamma-rays 400 cGy (41)	21 (51%)			20 (49%)
Gamma-rays 600 cGy (29)	19 (66%)	3 (10%)	1 (3%)	6 (21%)
EMS (40)	0	2 (5%)		38 (95%)

PCR Deletion Screening at the GPT Locus in AS52 Cells

Mutagen (Mutants analyzed)	Total Gene Deletion	Partial Gene Deletion	Insertion	No Alteration
Spontaneous (43)	22 (51%)	1 (2%)	2 (4%)	18 (42%)
Gamma-rays 400 cGy (25)	25 (100%)			
X-rays 310 cGy (18)	14 (78%)			4 (22%)
EMS (51)	8 (14%)			43 (84%)

Investigation of Encapsulation of Diazoluminomelanin (DALM) in Liposomes

This report, which covers the period of 15 October 1991 to 1 April 1992, describes the progress in the encapsulation of diazoluminomelanin (DALM) into liposomes for the ultimate incorporation into mammalian cells. Previous attempts to incorporate DALM into cells either by endocytosis, phagocytosis or lipofection have failed. The major reason for this was thought to be due to the fact that DALM is a highly anionic polymer (much like DNA) and is repulsed from the negatively charged cell.

Dr. Johnathan Kiel of the Radiofrequency Radiation Division, U.S. Air Force Armstrong laboratory, provided the samples used for these experiments. The samples included approximately 1g of chemically synthesized DALM, 500 mg truncated DALM and 10 mg poly diazoaminotyrosine (poly DAT) which was synthesized in the absence of luminol. These compounds, along with the monomer 3-aminotyrosine purchased from Sigma, were used to make liposomes. Each compound was dissolved in phosphate buffered saline (PBS), Ph 7.4, and titrated to Ph. 7.4 with either acid or base. The one exception was 3-amino tyrosine, which precipitated at pH 7.4 and was used at pH 5.0. Figure 1 depicts the absorbance spectrum of each of these compounds in PBS, pH 7.4. As previously described, DALM produced a broad spectrum. There was little difference in the spectrum of DALM and truncated DALM. The poly DAT spectrum was broad in the visible region, with a similar peak signature to 3-amino tyrosine in the ultraviolet region. Both DALM and truncated DALM solutions were purple and were stable for weeks. 3-amino tyrosine was yellow but turned brown within 24 hours. Poly DAT was a black solution.

Liposome Formation

First, liposomes containing DALM were made by rehydrating a thin lipid film of distearoyl phosphatidylcholine (DSPC) and cholesterol (Chol). Samples were also made with these lipids plus the negatively charged lipid, dimyristoyl phosphatidylglycerol (DMPG). These liposomes were formed in both water and PBS, pH 7.4. All liposome samples were observed as multilamellar structures under the light microscope. To determine the encapsulation efficiency, an aliquot of each liposome sample was spun at 13,000 x g. The liposomes pelleted. The supernatant was scanned in the spectrophotometer. The absorbance profile was compared to that of the original solution used to rehydrate the lipid mixture. A decrease in the absorbance at 260nm of the supernatant would be observed if encapsulation or association occurred.

The encapsulation of DALM was also checked by scanning the liposome samples before and after centrifugation against blank liposomes of the same composition. In each case, the liposomes caused too much light scattering.

By either technique the encapsulation efficiency was low. Samples were also made by rehydrating the lipid mixture in PBS, pH 7.4, containing 1M NaCl to displace the charge effect between the anionic DALM polymer and the liposomal surface. Also the lipid mixtures were subjected to five (5) freeze-thaw cycles prior to lyophilization and rehydration. This latter technique has been found to improve encapsulation efficiency. None of these changes caused a significant increase in the amount of DALM encapsulated.

Although the actual structure and molecular weight of DALM is not known, it is thought to be a large polymer. To determine if the highly negative charge or large size affected the encapsulation efficiency of DALM into liposomes, neutral, negatively charged and positively charged liposomes were made using

stearyl amine. The different charged liposomes were formed by rehydrating freeze-dried lipid mixtures with either PBS, 3-amino tyrosine (3AT), poly DAT, truncated DALM and DALM. Figures 2A-2D describe the results of these experiments. In each case, very little encapsulation was observed. The absorbance profiles of the original solution and the supernatant were very similar. These results suggested that the liposome composition may be the problem, particularly when little encapsulation was noted for the small molecule, 3-amino tyrosine.

Cholesterol was removed from the composition. Multilamellar liposomes containing only DSPC or DSPC: stearyl amine (9:1) of either PBS, 3-amino tyrosine or DALM were made. The results are shown in Figures 3A and 3B. The samples showed higher encapsulation efficiencies than previous samples. The DSPC sample containing 3-amino tyrosine was yellow. The DALM liposome pellets were purple for both lipid compositions. Since the original composition contained approximately 40 mole % cholesterol, the liposomes may have been too rigid to allow passage of such a large polymer.

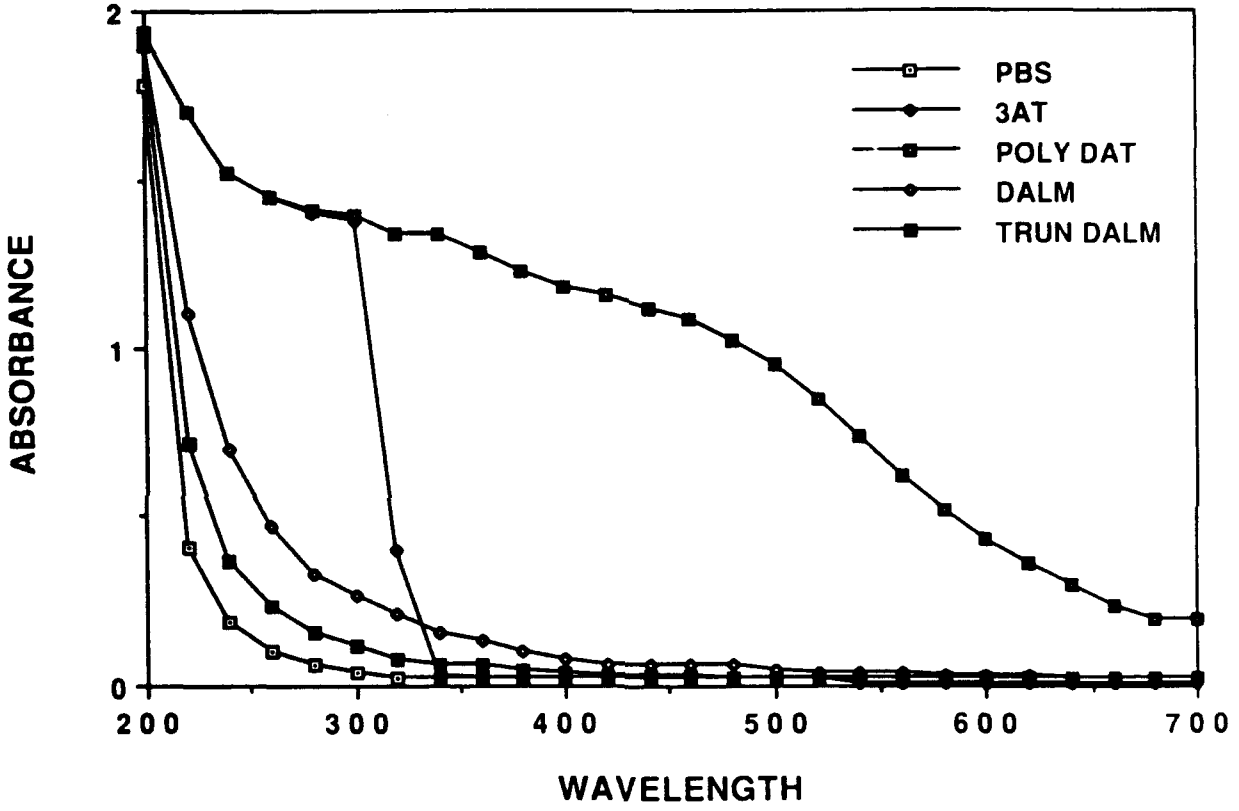
Future Directions

Samples containing less cholesterol should be made. Samples with cholesterol are less susceptible to fusion upon storage and are more stable in serum. Once an acceptable composition is found, the incorporation of the liposomes into cells should be attempted.

Lastly, experiments using the bacterially produced DALM should be carried out, since no more chemically synthesized DALM is available. Dr. Kiel now has DALM produced in Bacillis anthracis, grown by fermentation in liter quantities. Although there appear to be differences in the chemical reactivity and microwave

absorption properties of DALM produced by the two techniques, both are large polymers and probably will not be readily taken up by cells. Both DALM released by the bacteria into the fermentation broth and isolated from whole frozen cells should be used for the liposome encapsulation experiments. These studies may lead to a better understanding of the structure of DALM, as well as provide more information about the location of DALM synthesis in the bacteria.

Figure 1


2
1
0
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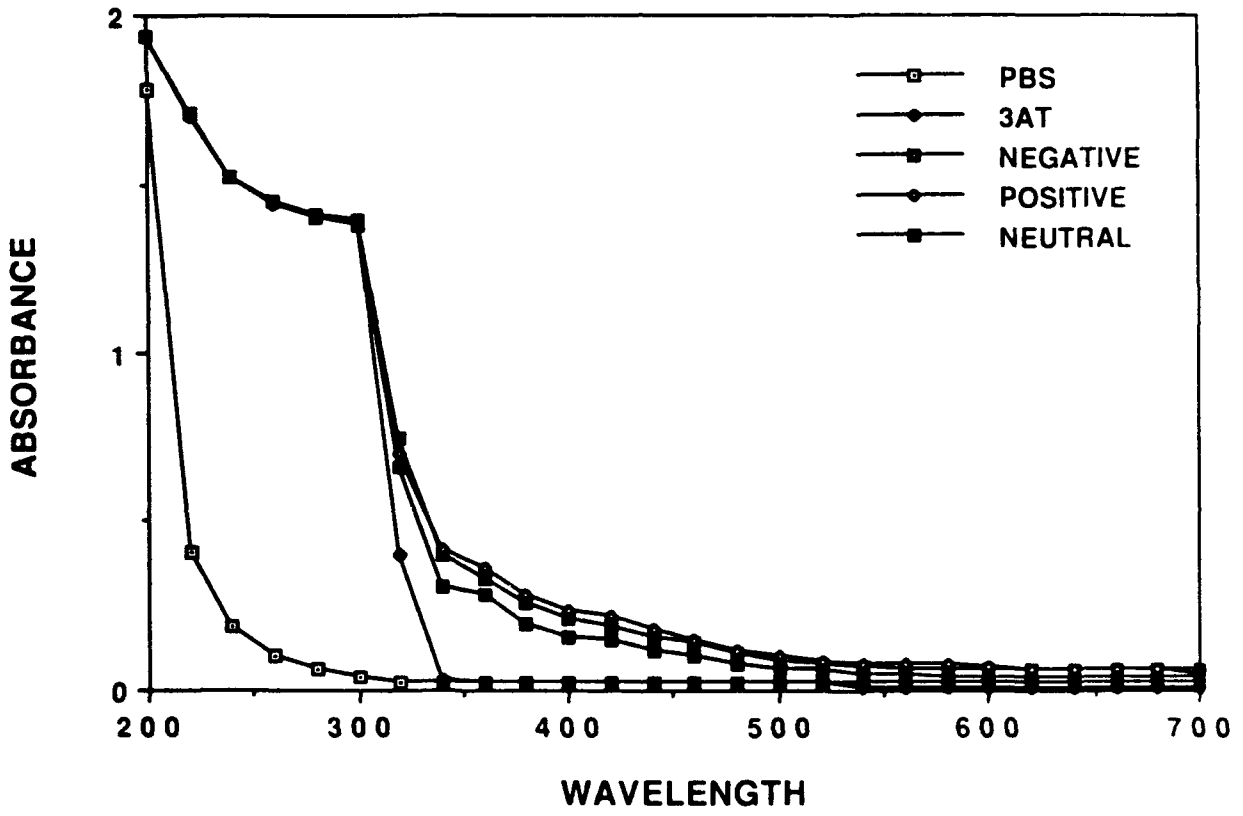
400
500
600
700
WAVELENGTH
PBS
3AT
POLY DAT
DALM
TRUN DALM

Figure 2A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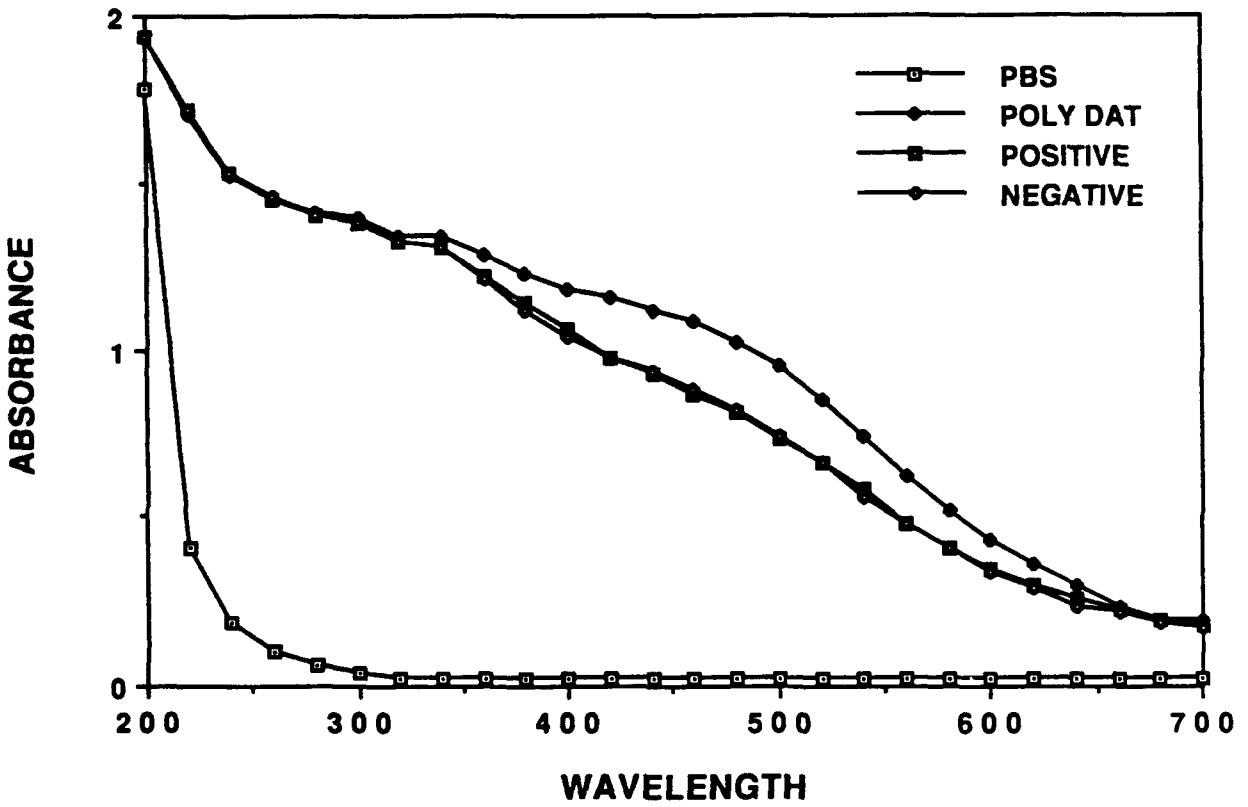
NEUTRAL

Figure 2B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WAVELENGTH

Figure 2C

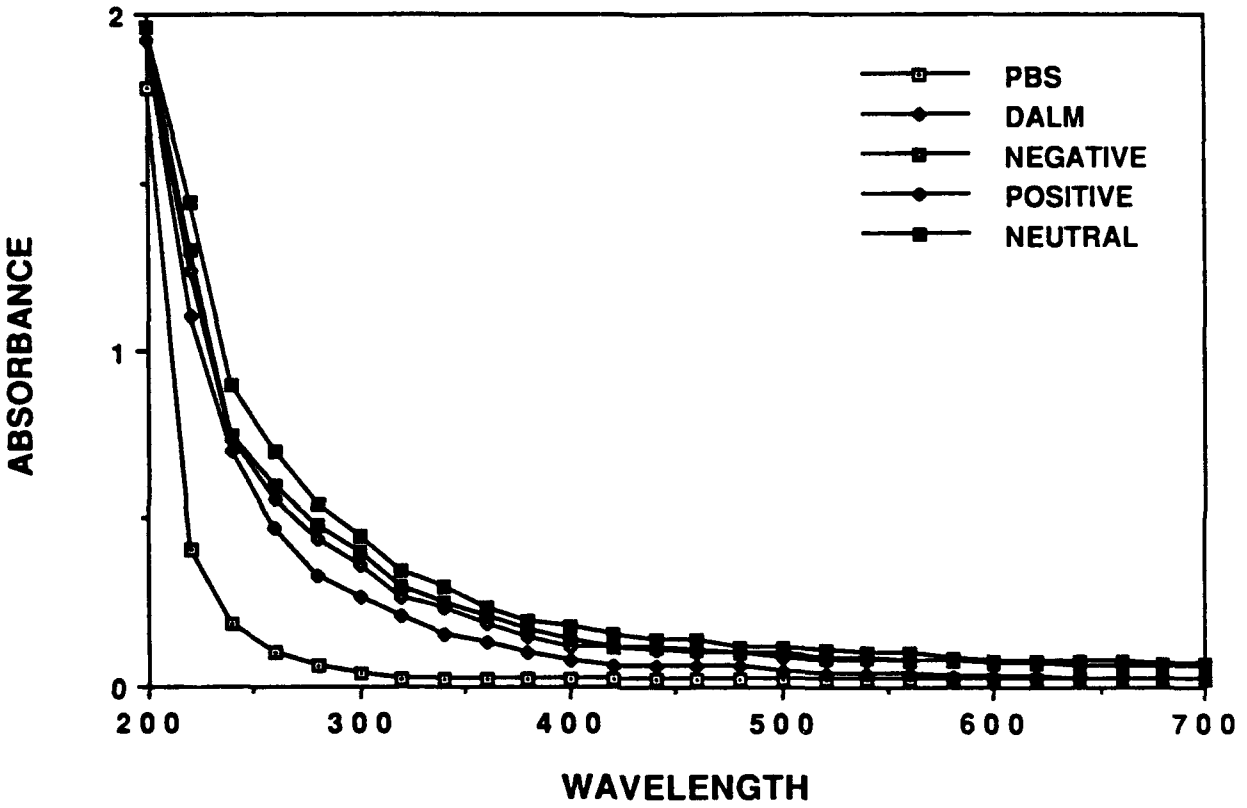
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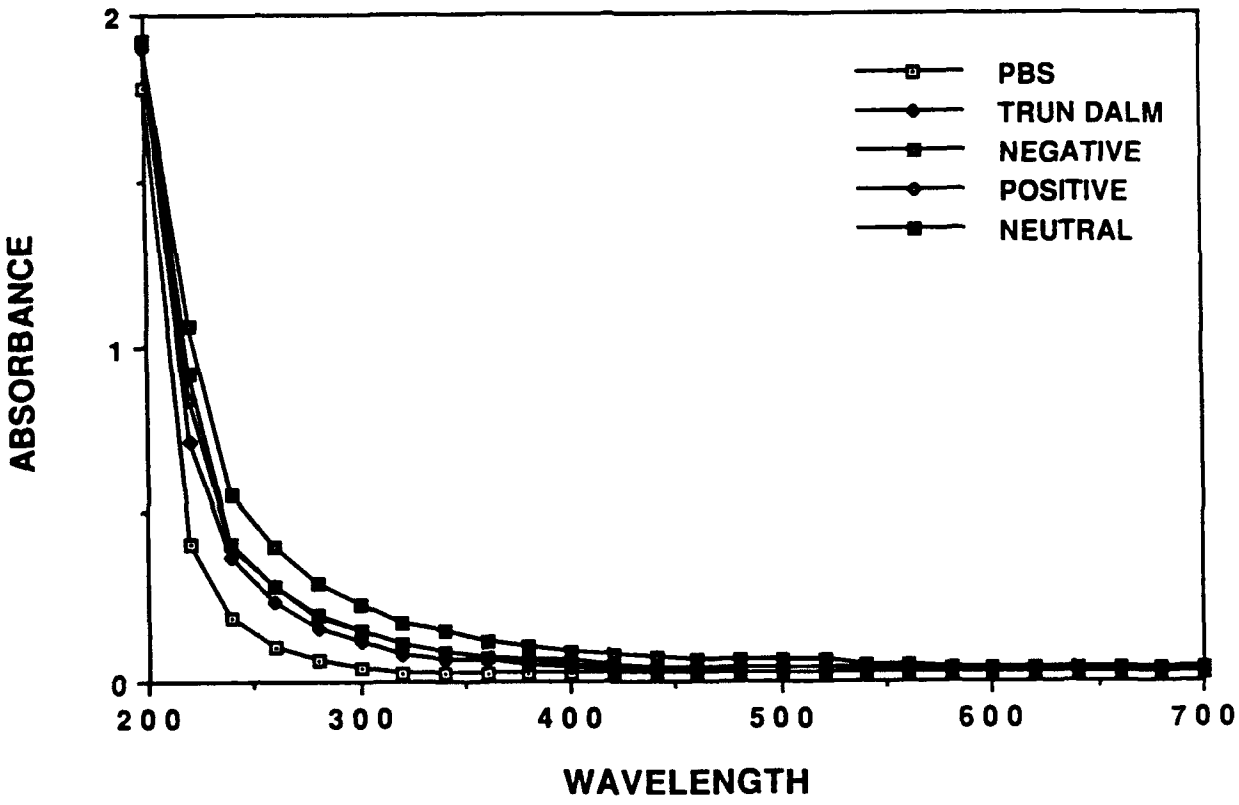
Figure 2D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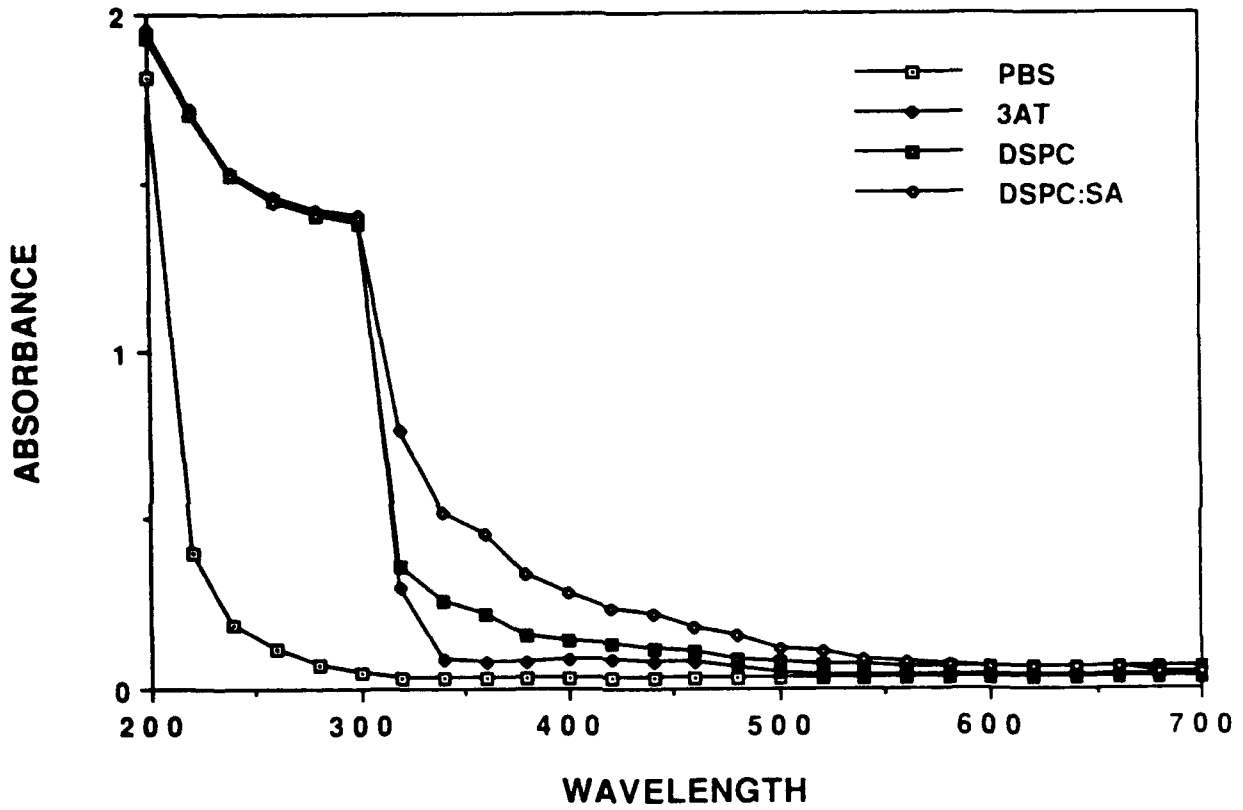
TRUN DALM
NEGATIVE
POSITIVE
NEUTRAL

Figure 3A


2
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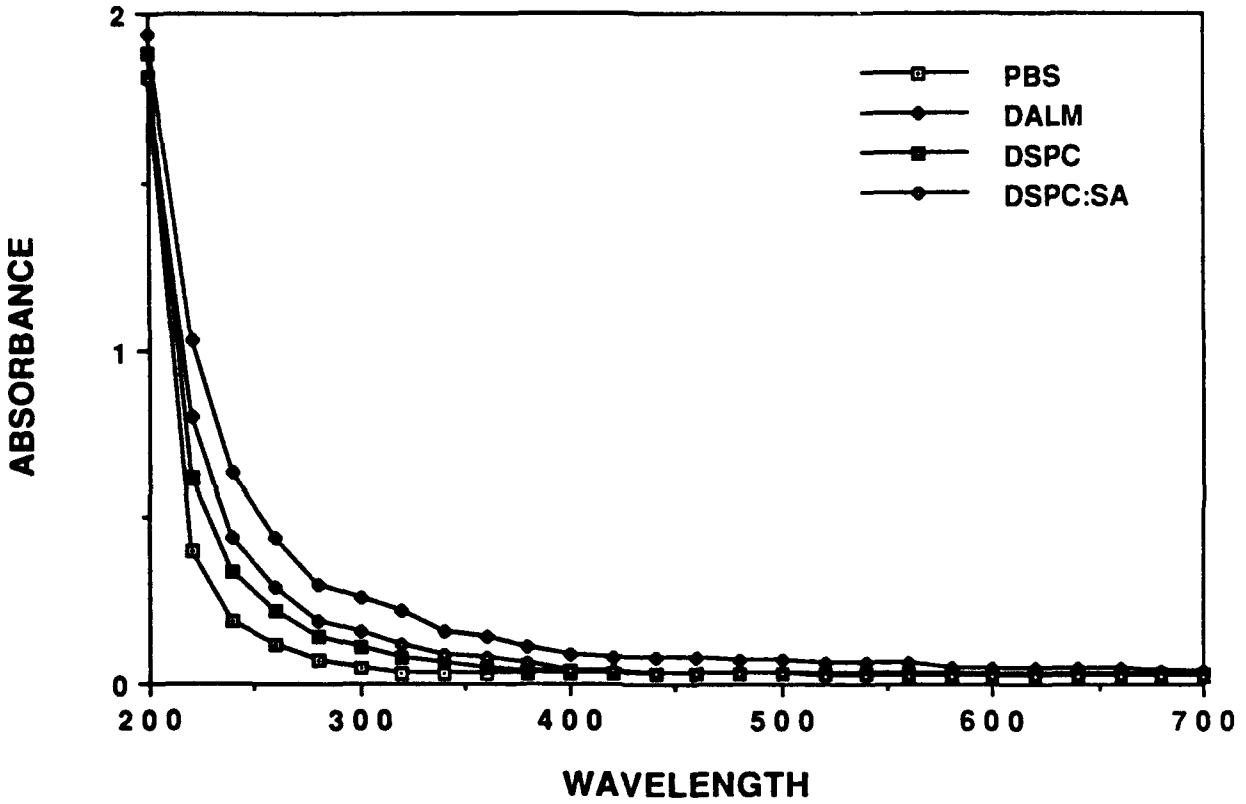
500
600
700
WAVELENGTH
PBS
3AT
DSPC
DSPC:SA

Figure 3B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WAVELENGTH
PBS
DALM
DSPC
DSPC:SA

Monitoring Intracellular Metabolism in Cells Using Nuclear Magnetic Resonance Spectroscopy

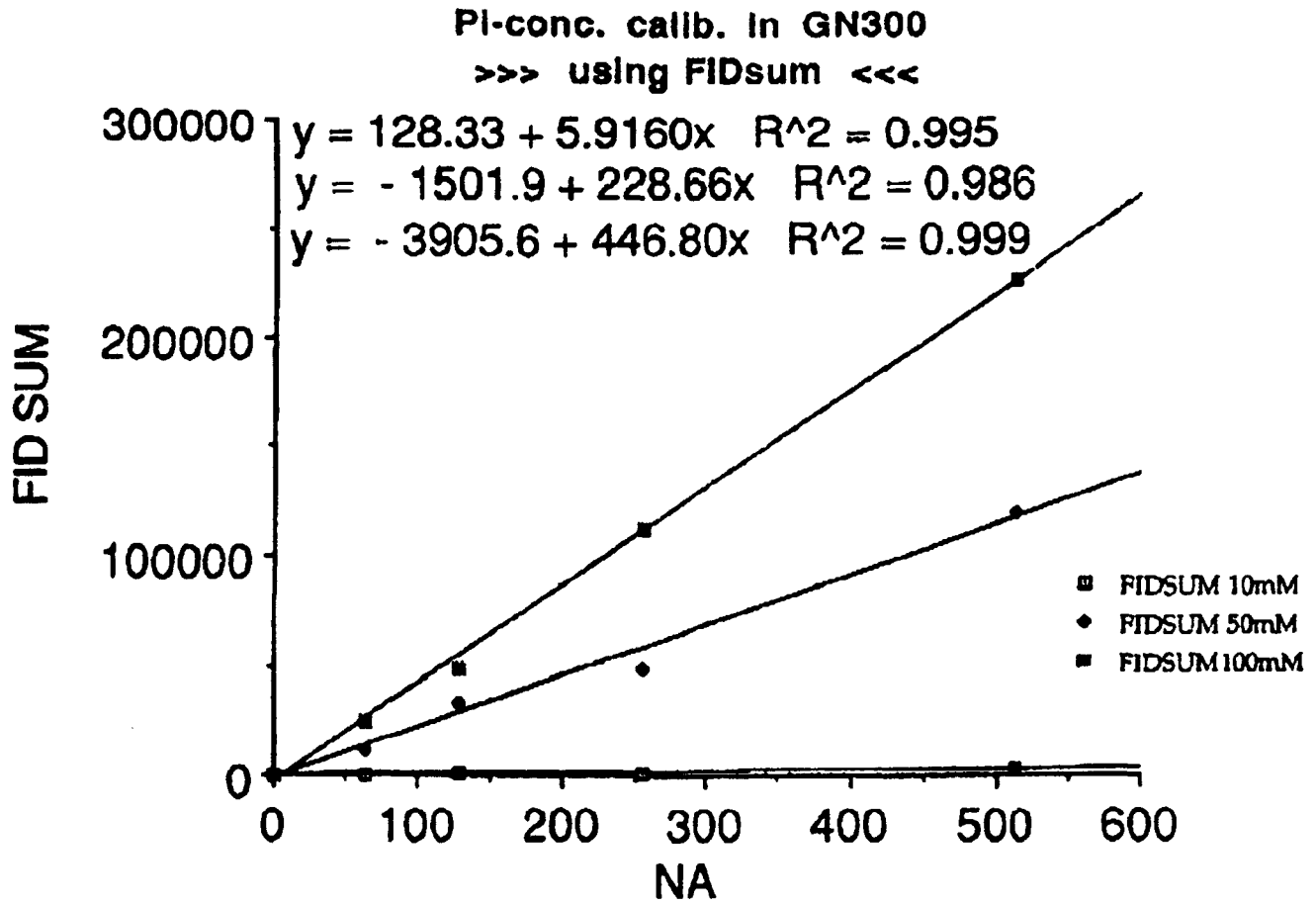
Phosphorous (^{31}P) nuclear magnetic resonance (NMR) spectroscopy has been used to monitor cellular metabolism and energetics (Jacobsen and Cohen 1981, Nicolay et al. 1983). Some *in vitro* methods have typically employed perchioric acid extracts of cells (Navon et al., 1977); this method however, involves disruption of the cells and is not suitable for a dynamic study of cell metabolism. Other *in vitro* NMR studies, using intact cells, require large numbers of cells (~10^8 per ml) for spectra with good signal-to-noise ratios. Cells at this high density are packed in a nonphysiologic state, which eventually results in death of the cells due to the accumulation of toxic metabolites. In order to study cells in a system which more closely approximates an *in vivo* NMR study, we are adapting techniques which will improve the biological environment by using agarose gel threads to suspend the cells. This will allow for adequate perfusion of fresh media to prevent a buildup of toxic metabolites (Foxall and Cohen, 1983).

This study will embed human lymphoblast 244B cells in agarose gel threads to allow monitoring of the dynamic phosphate metabolism following exposure to microwave radiation (2.45 GHz), alone or in combination with X-rays. Data will be acquired on a General Electric GN-300 (7 Tesla) nuclear magnetic resonance spectrometer, which is a wide bore (89 mm), fully broad-banded spectrometer with a variable temperature capability. The Research Imaging Center, Magnetic Resonance Spectroscopy Division, has 7 probes for the GN-300. The 10 mm N^{15}–P^{31} broad-band probe tuned to ^{31}P is being used in this study with a 10 mm NMR tube equipped with a specially designed insert to allow for cell perfusion while under controlled temperature conditions in the bore of the magnet.

Preliminary Results

Before employing the agarose thread perfusion method, a pilot study using only cells in suspension was performed. Several samples of cells ranging from 10^7 to 10^8 were washed with phosphate free media and examined by ^{31}P NMR. Initially, the number of transients (spectral acquisitions) collected for each experiment was varied. These experiments were done to establish the experimental parameters for optimum data acquisition while the perfusion apparatus was being machined. Additional experiments using inorganic phosphate at various concentrations close to what is expected in the cell mixture were run. Figures of these data are attached. These control experiments have established optimum instrument parameters and data treatment for subsequent use in the gel experiments. It was also necessary to determine the time course of the NMR experiments to plan the microwave protocols for later experiments. Preliminary experiments used three concentrations of inorganic phosphate and measured the signal intensity for the number of acquisitions (NA) ranging from 75 to 510. Figure 4 measures the intensity by summing the free induction decay (FID), while Figure 5 compares the intensities by measuring the area under the peak. The FID sum gives more reliable measure of the total inorganic phosphate in these controls. Graph 6 compares the signal-to-noise ratio for these same samples. The inorganic phosphate experiments have been used to estimate the number of acquisitions required for acceptable signal-to-noise ratio for the cell experiments. Other instrumental parameters, such as RF flip angle, decoupling parameters, etc. have been established for control phantoms. As soon as cells imbedded in agarose are available, ^{31}P-NMR will establish the optimum conditions for the perfusion experiment. Once established, these instrumental settings will be employed for all subsequent data collection.

Figure 4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y = - 1501.9 + 228.66x R^2 = 0.986
y = - 3905.6 + 446.80x R^2 = 0.999
FID SUM
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200000
100000
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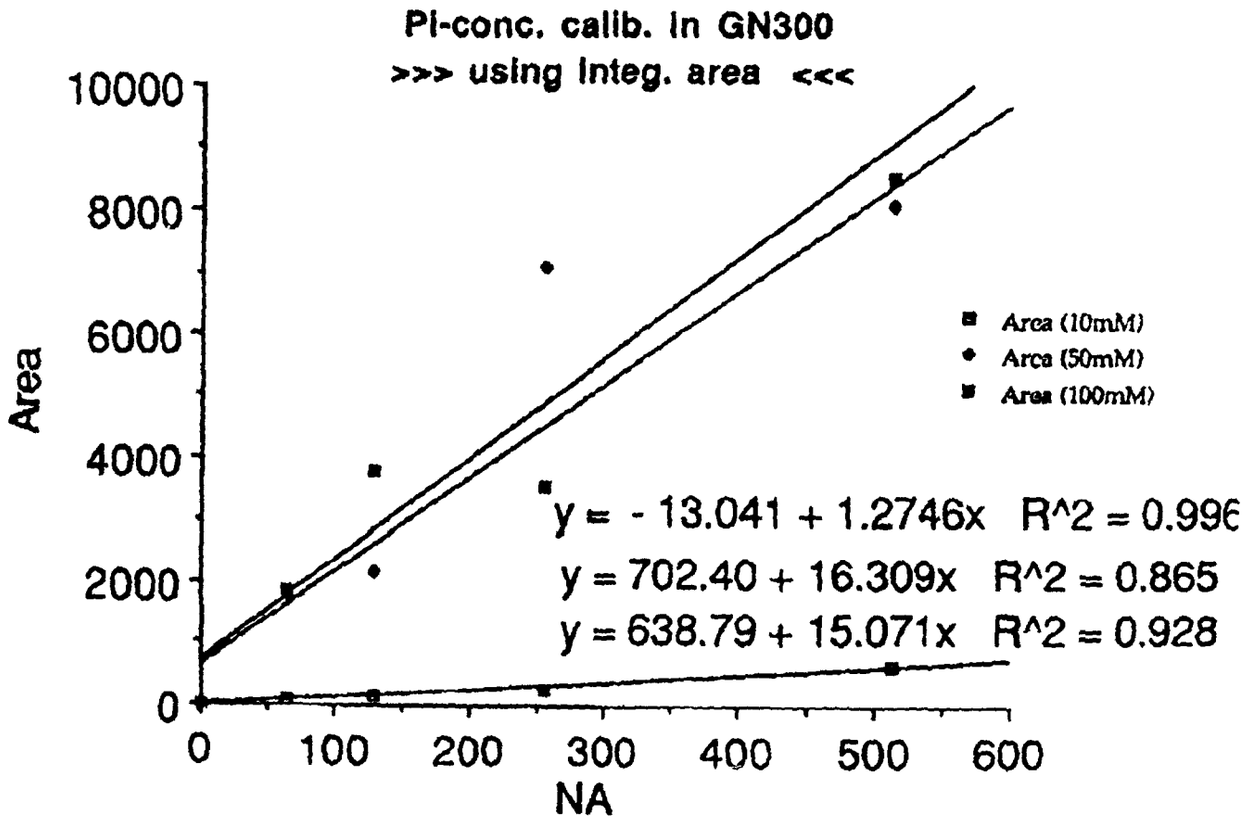
100
200
300
400
500
600
NA
FIDSUM 10mM
FIDSUM 50mM
FIDSUM 100mM

Figure 5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Area (10mM)
Area (50mM)
Area (100mM)
y = - 13.041 + 1.2746x R^2 = 0.996
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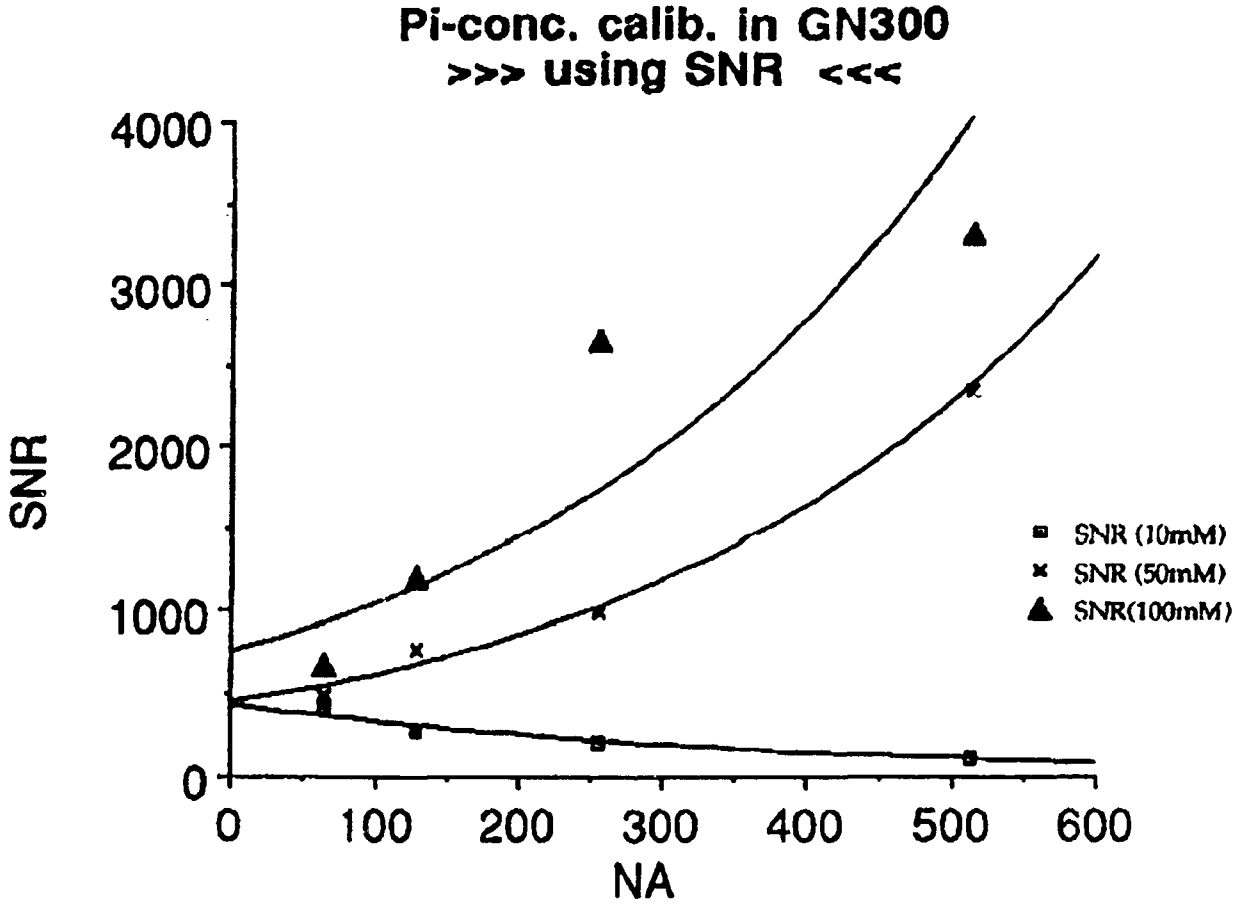
Figure 6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1000
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100
200
300
400
500
600
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SNR (10mM)
SNR (50mM)
SNR(100mM)

REFERENCES

1. Jacobson L, and Cohen JS (1981) Biosci Rep 1:141–150.

2. Scheffers WA, Bruinenberg PM, Koptein R (1983) Archives of Microbiol 134:270–275.

3. Navon B, Ogawa S, Shulman RG, Yamane T (1977) Proc of the Nat Acad of Sci USA 74:87–91.

4. Foxall D, and Cohen JS (1983) J Magn Reson 52:346.

5. Foxall D, Cohen JS, Mitchell JB (1986) Exp Cell Res 154:521.

6. Daly PF, Lyon RC, Straka E, and Cohen (1988) FASEB J 2:2596.

4. Articles Submitted for Publication

Hsie AW, Xu Z, Yu Y An J, MELTZ ML, Schwartz JL, and Hrelia P (1992) Quantitative and Molecular Ananyses of Genetic Risk: A Study with Ionizing Radiation

5. Participating Professionals

The professionals participating in the first year's activities of this research project included:

Principal Investigator

Martin L. Meltz, Ph.D.
Associate Professor of Radiology
Division of Radiation Oncology
University of Texas Health Science Center at San Antonio

NMR Spectroscopy Investigation

Lou Jean Floyd, Ph.D.
Lecturer/Instructor
University of Texas Health Science Center at San Antonio
Research Imaging Center

Robert F. Williams, Ph.D.
Associate Professor
University of Texas Health Science Center at San Antonio
Research Imaging Center

Liposome Studies

Beth A. Goins, Ph.D.
Clinical Assistant Professor
Division of Nuclear Medicine
University of Texas Health Science Center

Mutation Spectrum Analysis

Abraham W. Hsie
Professor
Dept. of Preventive Medicine & Community Health
University of Texas Medical Branch (UTMB) at Galveston

6. Interactions with U.S. Air Force Personnel

Margaret Baker, Ph.D., of the Department of Radiation Oncology, School of Medicine, University of Pennsylvania, visited the UTHSCSA as part of the project on 17 and 18 May, 1991. She presented a seminar at the UTHSCSA entitled "Oxidation and Radiation." The staff members of the Radiofrequency Radiation Branch at USAF Armstrong Laboratory were invited to hear her talk.

Abraham Hsie, Ph.D., of the Department of Preventive Medicine and Community Health, UTMB, Galveston, visited the UTHSCSA on 12 and 13 June, 1991. As part of this project, Dr. Hsie presented a seminar at the Directed Energy Division, USAF Armstrong Laboratory, on 13 June, 1991. The talk was entitled: "Molecular Mutagenesis and Genetic Risk of Ionizing Radiations." The POC was Dr. Johnathan Kiel.

Joseph C. K. Liu, Graduate student, Department of Radiology and Radiation Biology, Colorado State University, visited the UTHSCSA on 15 and 16 August, 1991. During his visit, as part of this project, Mr. Liu presented a seminar at USAF Armstrong Laboratory entitled "Effects of Amiloride on the regulation of intracellular pH and thermotolerance in CHO cells."

In addition to the formal interactions described above, Dr. Meltz had frequent telephone conversations during the course of the year with Dr. Johnathan Kiel at Armstrong Laboratory, and made a few informal visits to the laboratory. On two occasions, Dr. Meltz was accompanied by Dr. Beth Goins; they participated in several hours of scientific dialogue with Dr. Kiel concerning the properties of DALM and Dr. Goins' efforts to encapsulate DALM in liposomes. In addition, Dr. Meltz escorted three members of the Instrumentation Department at UTHSCSA to the Radiofrequency Radiation Division facility, to examine the existing thermal Control System prior to the final redesign of the instrumentation.

7. New Inventions

During the first year of this project, the decision was made to acquire a Thermal Control system similar to the original prototype constructed for the Radiofrequency Radiation Branch, USAF Armstrong Laboratory. The original design is under patent to the U.S. Air Force. With the approval of Dr. Johnathan Kiel, a redesign of the original invention and new software development (an improvement) was initiated during this first year at UTHSCSA as part of this AFOSR Grant. Part of the funds came from the Roy and Ellen Quillan Foundation; the remainder came from the budgeted first year funds of this project, after approval by AFOSR. The new design is being constructed by the Instrumentation Division of the UTHSCSA; in contrast to the original design, it allows for one controller unit to simultaneously control the temperature of liquid materials in two (2) different incubation chambers. In addition, because the original software language was unique and restrictive, the software is being completely and independently written by the personnel of the Department of Computing Resources of the UTHSCSA in a more versatile computer language. Because the design, software development, and construction of the system is not complete at the end of this project period, it is not detailed herein.

In addition to the Thermal Control System, a second invention is in the design stage. This invention would, if functional, support the mixing of cells in a test tube in a circular waveguide without any external agitation. The initial design took place after discussion of the concept with Dr. Johnathan Kiel, USAF Armstrong Laboratory.

8. Other Information

Staffing Information

Prior to the receipt of notification of award of this research project, two of the scientists available to participate in the project, Patricia K. Holahan, Ph.D. and Steven K. Smith, Ph.D., became unavailable for participation in the project. Dr. Holahan's husband, an officer in the U.S. Army, was transferred to Ft. Dietrick, Maryland; Dr. Holahan, an Assistant Professor, now has a position with the Nuclear Regulatory Commission. Dr. Smith, a Research Scientist, accepted an alternative position in a Radiation Oncology Research Project; he is no longer associated with the University.

As soon as the award became official, a national search was initiated to recruit two talented individuals to replace Dr. Holahan and Dr. Smith. This effort included listing of the positions with the placement services of several national scientific societies, including the Bioelectromagnetics Society, the Radiation Research Society, and the Environmental Mutagen Society. Letters were also written to scientific colleagues, Chairmen of radiation related departments and Directors of Radiation Oncology programs at universities and research laboratories across the country. Personnel recruiting also took place at the International Congress of Radiation Research. Appropriate individuals to replace Drs. Holahan and Smith were not available during the first year of this project.

In order to accomplish the research desired in the first year of this project, the decision was made to initiate a subcontract to Dr. Abraham Hsie of the University of Texas Medical Branch at Galveston. While Dr. Hsie had previously agreed to be a consultant to this research, he subsequently supervised the development at his laboratory of special advanced techniques of mutation spectrum analysis. Dr. Hsie agreed to apply these techniques to an analysis of

spontaneous mutants arising in the BH4 and AS52 chinese hamster ovary cell lines, so that we would have baseline data as to the types of mutations which occur spontaneously. A report of his research can be found in the **Results** section of this report. The subcontract was initiated after approval was obtained from AFOSR.

In addition to the work of Dr. Hsie, discussions with Dr. Johnathan Kiel led to an interest in attempting to incorporate diazoluminomelanin (DALM) into liposomes. DALM is a unique microwave absorbing chemical created at Armstrong Laboratory. The ultimate goal was to use liposomes to deliver the chemical into mammalian cells. Dr. Beth Goins of the Division of Nuclear Medicine, Department of Radiology, UTHSCSA, is an expert in liposome encapsulation; she agreed to perform these investigations. This was done with Dr. Kiel's agreement, as part of this research project. A complete report of Dr. Goins' work and suggestions appears in the **Results** section.

As the first year drew to a close, correspondence had already been initiated with two scientists who were prospective candidates for the open positions; Dr. Adapa Prasad of the University of Rochester, New York, and Dr. Sandra Schneider, of the UTHSCSA.

Additional Outside Funding

During the course of the project, application was made to a private non-profit foundation for funding to be applied toward the construction of a Thermal Control System. The funding was approved, in the amount of $7,600., from the Roy and Ellen Quillen Foundation.